ab214025 – Human IL-1 beta ELISA Kit

For the quantitative measurement of IL-1 beta in human serum, plasma, and cell culture supernatant samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: **www.abcam.com/ab214025**

Storage and Stability: Store kit at 2-8ºC immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 wells	Quantity 10 x 96 wells	Storage Condition
Human IL-1 beta Capture Antibody 10X	600 µL	10 x 600 µL	+4ºC
Human IL-1 beta Detector Antibody 10X	600 µL	10 x 600 µL	+4ºC
Human IL-1 beta Lyophilized Recombinant Protein	2 Vials	10 x 2 vials	+4ºC
Antibody Diluent 4BI	6 mL	10 x 6 mL	+4ºC
Sample Diluent NS	50 mL	2 x 250 mL	+4ºC
Wash Buffer PT 10X	20 mL	200 mL	+4ºC
TMB Development Solution	12 mL	120 mL	+4ºC
Stop Solution	12 mL	120 mL	+4ºC
Pre-Coated 96-Well Microplate	96 Wells	10 x 96 Wells	+4ºC
Plate Seal	1	10	+4ºC

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- – Microplate reader capable of measuring absorbance at 450 or 600 nm.
- – Method for determining protein concentration (BCA assay recommended).
- – Deionized water.
- – Multi- and single-channel pipettes.
- – Tubes for standard dilution.
- – Plate shaker for all incubation steps.
- – Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

- – Equilibrate all reagents to room temperature (18-25ºC) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.
- – Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.
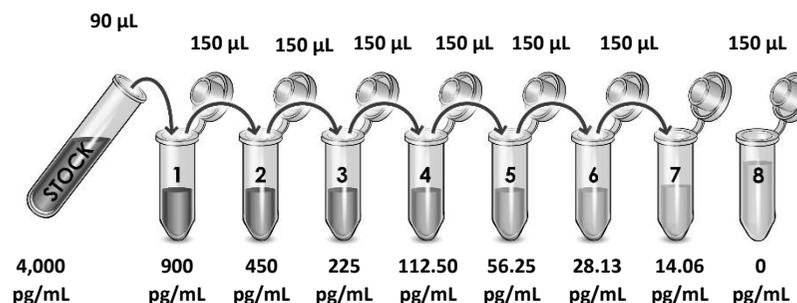
Version 5a, Last updated 13 September 2024

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 4BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 4BI. Mix thoroughly and gently.

Standard Preparation

- – Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).
- – **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the IL-1 beta standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the IL-1 beta standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 4,000 pg/mL **Stock Standard** Solution.

1) Label eight tubes, Standards 1– 8.
2) Add 310 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
3) Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

	90 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL	150 µL
STOCK	1	2	3	4	5	6	7	8
4,000 pg/mL	900 pg/mL	450 pg/mL	225 pg/mL	112.50 pg/mL	56.25 pg/mL	28.13 pg/mL	14.06 pg/mL	0 pg/mL

Sample Preparation

TYPICAL SAMPLE DYNAMIC RANGE	
Sample Type	**Range**
Serum*	≤ 50%
Plasma – Citrate*	≤ 50%
Plasma – Heparin*	≤ 50%
Plasma – EDTA*	≤ 50%
Cell culture supernatant	Varies by type

*Based on spiked sample.

Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1: 2 into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1: 2 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants: Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Or dilute samples as needed into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Plate Preparation
– The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
– Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
– For each assay performed, a minimum of two wells must be used as the zero control.
– For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
– Differences in well absorbance or "edge effects" have not been observed with this assay.

Assay Procedure
– Equilibrate all materials and prepared reagents to room temperature prior to use.
– We recommend that you assay all standards, controls and samples in duplicate

1) Prepare all reagents, working standards, and samples as directed in the previous sections.
2) Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3) Add 50 µL of all sample or standard to appropriate wells.
4) Add 50 µL of the Antibody Cocktail to each well.
5) Seal the plate and incubate for **2 hours** at room temperature on a plate shaker set to 400 rpm.
6) Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7) Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. *Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.*
8) Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9) Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength	600 nm
Time	up to 20 min
Interval	20 sec - 1 min
Shake	Shake between readings

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm

Calculation
1) Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
2) Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y axis) against the target protein concentration (x axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
   Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
3) Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
4) Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalysed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

Typical Data
Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.065	0.066	0.066
14.06	0.101	0.100	0.101
28.13	0.134	0.133	0.133
56.25	0.209	0.208	0.208
112.5	0.345	0.341	0.343
225	0.714	0.694	0.704
450	1.383	1.479	1.431
900	2.822	2.907	2.864

Figure 1. Example of human IL-1beta standard curve. The IL-1beta standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Typical Sample Values

Sensitivity

The calculated minimal detectable dose (mdd) is 5.64 pg/ml. The mdd was determined by calculating the mean of zero standard replicates (n=24) and adding 2 standard deviations then extrapolating the corresponding concentration.

Recovery

Three concentrations of IL-1beta were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
Human Serum	103	101 – 105
Human Plasma – Citrate	86	84 – 88
Human Plasma – Heparin	100	99 – 101
Human Plasma – EDTA	93	90 – 96
PBMC + PHA Supernatant	98	96 – 100

Linearity of dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native IL-1beta was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	50% PBMC + PHA Supernatant	100% THP-1 + LPS Supernatant
Undiluted	pg/mL	637	718
	% Expected value	100	100
2	pg/mL	303	351
	% Expected value	95	98
4	pg/mL	151	174
	% Expected value	95	97
8	pg/mL	81	102
	% Expected value	101	114
16	pg/mL	47	NL
	% Expected value	119	NL

NL – Non-Linear

Recombinant IL-1beta was spiked into the following biological samples and diluted in a 2-fold dilution series in Sample Diluent NS.

Dilution Factor	Interpolated value	50% Human Serum	50% Human Plasma (Citrate)	50% Human Plasma (EDTA)	50% Human Plasma (Heparin)
Undiluted	pg/mL	444	385	445	451
	% Expected value	100	100	100	100
2	pg/mL	232	207	228	221
	% Expected value	104	107	103	98
4	pg/mL	114	105	116	115
	% Expected value	103	109	104	102
8	pg/mL	56	53	57	58
	% Expected value	101	110	103	103
16	pg/mL	24	26	27	27
	% Expected value	87	107	96	97

Precision

Mean coefficient of variations of interpolated values of IL-1beta in 3 concentrations of PMBC + PHA supernatant within the working range of the assay.

	Intra-Assay	Inter-Assay
n=	8	3
CV (%)	4.8	5.6

Assay specificity

This kit recognizes both native and recombinant human il-1beta protein in serum, plasma, and cell culture supernatant samples only.

Cell and tissue extract samples have not been tested with this kit.

Cross reactivity

Recombinant mouse il-1beta and human il-1 receptor antagonist were prepared at 50 ng/ml and 225 pg/ml and assayed for cross reactivity. No cross-reactivity was observed.

Interference

Recombinant human interleukin-1 receptor type 1 was prepared at 50 ng/ml and 225 pg/ml and tested for interference. No interference with was observed.

Species reactivity

This kit recognizes human il-1beta protein.

Other species reactivity was determined by measuring serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:
- Mouse
- Rat
- Cow

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide